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CHERESKIN, C	

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NICHOLAS J. SEAY
QUARLES & BRADY
FIRST WISCONSIN PLAZA
P.O. BOX 2113
MADISON, WI 53701-2113

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☑ This application has been examined ☑ Responsive to communication filed on 8/30/90 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire three(3) month(s), _____ days from the date of this letter. Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☑ Notice of References Cited by Examiner, PTO-892. ??
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☑ Claims 1-17 are pending in the application.

   Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☑ Claims 1-17 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☑ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).

12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.

13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

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EXAMINER'S ACTION

PTOL-326 (Rev.9-89)

Claims 1-16 and new claim 17 remain.

The text of those sections of Title 35 U.S. Code not included in this action can be found in a prior Office action.

The rejection under 35 U.S.C. 112 second paragraph on page 2 of the previous office action is withdrawn in view of Applicants' amendments.

The rejection under 35 U.S.C. 112 first paragraph on page 2 of the previous office action is withdrawn in view of Applicants' arguments.

The specification is newly objected to under 35 U.S.C. 112, first paragraph, as failing to provide an adequate written description of the invention, and failing to adequately teach how to make and/or use the invention. Applicants have not demonstrated that the synthetic gene exemplified or any other synthetic gene has insecticidal activity. The data set forth in Table 1 indicates that constructs with modified sequences based upon codon usage do not work much better than the control. Applicants recite that "the synthetic sequences exhibited a much more uniform and greater toxicity to hornworms" (present specification, page 19, lines 18-19). However, it is not clear how this conclusion was reached. The data of Table I indicates high variability, but also that the control was in the same range as plants transformed with synthetic gene constructs (25%-83% for synthetic constructs versus 39% for control). In other words, pTVAMVBT3 and pTVAMVBT4 had kill rates of 46% and 25%, respectively, which appears to be similar to the 39% level for the control. pTVAMVBT2 was significantly higher at 83%. If this construct works consistently better, Applicants may wish to consider limiting their claims to this construct. However, with this degree of variability, Applicants' disclosure is not enabling for the broader claims recited and is an "invitation to experiment" in regard to applying the guidelines provided by Applicants to variations falling within the scope of the present claims.

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Although, the distribution of clones into the "9" rating is slightly higher for the synthetic constructs, it is not clear what criteria were used to establish the various categories 6-9. Furthermore, since the majority of the control plants fell into the "8" category, the relevant question, which does not appear to be addressed in the specification, is whether or not the difference between an "8" and a "9" rating is significant. Moreover, even if these deficiencies were remedied, the specification would only be considered enabling for claims limited as set forth below.

Claims 1-17 are newly rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

Claims 1-17 are rejected under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited to Manduca sexta and other insect species which can be reasonably extrapolated from the teachings of the examples in the specification. See MPEP 706.03(n) and 706.03(z).

A given B. t. toxin does not show toxicity against all insect species (See Hofte et al, Table 5; Vaeck et al, page 37, column 1, for example). Thus, enablement for Applicants' claims appears to be limited to the insect species tested.

Claims 1-17 are rejected under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited to tobacco and other plant species which can be reasonably extrapolated from the teachings of the examples in the specification. See MPEP 706.03(n) and 706.03(z).

Applicants have only demonstrated the effectiveness of the claimed method in tobacco and it is known that insecticidal proteins may be expressed differently in different plant species. For example, Vaeck et al and Barton et al teach that a full length Bt containing construct in tobacco was ineffective whereas Fischhoff et al reported that constructs containing full length Bt sequences were effective in tomato. Thus, there is reason to

believe that gene expression of insecticidal proteins will vary as a function of the specific plant species.

Claim 1-17 are rejected under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited to dicot cells. See MPEP 706.03(n) and 706.03(z).

There is no evidence in the specification that the claimed invention is enabled for plant species in general. Plant species vary with respect to their ability to undergo transformation and regeneration. It is noted that regeneration of monocot plants was not a routine procedure at the time of filing 07/390,561. See Potrykus, for a discussion of the state of the art with respect to monocot transformation and regeneration in 1990. Consequently, enablement for Applicants' claimed invention is limited to dicot species.

Claims 1-17 are rejected under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited to claims which recite the upper sequence shown in Figure 2. See MPEP 706.03(n) and 706.03(z).

Applicants have shown only one B. thuringiensis derived synthetic gene which sequence is shown in Figure 2. In view of the unpredictability of expression of foreign genes, it does not appear that any synthetic gene which was functionally equivalent to any Bt toxin protein would be effective in any plant cell against any species of insect or that the method is broadly applicable to any foreign gene expressed in a plant cell as broadly claimed. As Applicants point out themselves, the pattern of codon usage is only one of the mechanisms to which poor expression may be ascribed (Amendment A, page 5, paragraph 2).

Claims 1-16 remain and new claim 17 is rejected under 35 U.S.C. 103 as being unpatentable over Hoekema et al taken with Murray et al, Schnepf et al, Vaeck et al, Hollenberg et al, and Seeburg et al as applied in the previous office action. Hoekema et al teaches a method of affecting gene

expression by exploiting codon usage. As recited in the previous office action, Hoekema et al disclose that when foreign proteins are used in yeast vector systems the expression level may decrease one or two orders of magnitude. Hoekema et al teach that the codon choice pattern was one parameter affecting this low level of expression and teaches that expression of native highly expressed yeast genes can be altered by substituting the codons usually found in yeast genes with minor codons which never or rarely occur in highly expressed natural genes . The amino acid sequence was not disturbed. As a result, both mRNA and protein synthesis were decreased.

Hoekema et al differ from the claimed invention primarily in that their work was directed to yeast cells, not plant cells as in the claimed invention. However, information on the codon usage in plants has been extensively reviewed. In a recent review, Murray et al teach that plant genes in general were known to be highly GC rich, especially in the third base as compared to procaryotic sequences which have a strong bias towards use of A or T in the third base. More specifically, in Bacillus thuringiensis as reported by Schnepf et al, it was known that the use of A or T was preferred. Additionally, it was known that B. thuringiensis toxins in particular were poorly expressed in plant cells as reported for example by Vaeck et al. Furthermore, Murray et al suggested the modification of the primary nucleic acid structure based upon the codon usage to increase expression in plants. Thus, it would have been obvious to one of ordinary skill in the art that the concept taught by Hoekema et al to address the problem of poor expression of foreign genes in yeast could be applied to poor expression of foreign genes in plants.

Methods of transfer of foreign genes to plant cells and expression in plant cells were all well within the ordinary level of skill in the art. Methods of making long oligonucleotides were known in the art as taught by Hoekema et al, Hollenberg et al (Figure 9), and Seeburg et al (Figure 2).

The Declaration of Michael J. Miller is defective in that it is not signed by all of the inventors. Consequently, this rejection has been maintained. However, if the Declaration were signed by all of the inventors and resubmitted, this rejection would be withdrawn because the Murray et al reference could no longer be applied.

Consequently, the application of the teaching of Hoekema et al to expression of plant cells was well within the ordinary skill in the art at the time the claimed invention was made as adequately demonstrated by the secondary references. One of ordinary skill would have had a reasonable expectation of success in enhancing gene expression by substituting the codons employed by the native gene with codons used in the host cell in view of the teaching of the prior art. Thus the claimed invention as a whole was clearly prima facie obvious over the references, in the absence of sufficient, clear, and convincing evidence to the contrary.

A new ground of rejection is set forth below.

Claims 1-17 are rejected under 35 U.S.C. 103 as being unpatentable over Hoekema et al taken with Grantham et al, Schnepf et al, Vaeck et al, Barton et al, Hollenberg et al, and Seeburg et al. Hoekema et al teaches a method of affecting gene expression by exploiting codon usage. Hoekema et al disclose that when foreign proteins are used in yeast vector systems the expression level may decrease one or two orders of magnitude. Hoekema et al teach that the codon choice pattern was one parameter affecting this low level of expression and teaches that expression of native highly expressed yeast genes can be altered by substituting the codons usually found in yeast genes with minor codons which never or rarely occur in highly expressed natural genes . The amino acid sequence was not disturbed. As a result, both mRNA and protein synthesis were decreased.

Hoekema et al differ from the claimed invention primarily in that their work was directed to yeast cells, not plant cells as in the claimed invention. However, modification of a procaryotic sequence to optimize expression in a plant cell was well within the ordinary level of skill in the art as shown by Grantham et al (see especially, Tables 1 and 2). More specifically, in Bacillus thuringiensis as reported by Schnepf et al, it was known that the use of A or T was preferred. It was also known that B. thuringiensis toxins in particular were poorly expressed in plant cells as reported for example by Vaeck et al and Barton et al. Furthermore, Barton et al teach the expression of insect toxins in tobacco using the pAMVBTS vector. Thus, it would have been obvious to one of ordinary skill in the art that the concept taught by Hoekema et al in yeast could be applied to expression of foreign genes in plants by modification of known vectors for expression of Bt toxins such as those taught by Barton et al and Vaeck et al in accordance with the guidelines provided by Hoekema et al and Grantham et al.

Methods of transfer of foreign genes to plant cells and expression in plant cells were all well within the ordinary level of skill in the art. Methods of making long oligonucleotides were known in the art as taught by Hoekema et al, Hollenberg et al (Figure 9), and Seeburg et al (Figure 2).

Applicants argue that "the pattern of codon usage is only one of the mechanisms to which this poor expression level may be ascribed" (Amendment A, page 5, paragraph 2). However, taking together the teachings of Vaeck et al and Barton et al on the poor expression of Bt toxins in plants, the teachings of Grantham et al and Schnepf et al on differences in codon usage between plants and B. thuringiensis, and the teaching of Hoekema et al on affecting gene expression by substitution of equivalent codons, one of ordinary skill in the art could reasonably expect that alteration of the nucleic acid sequence to favorably effect codon usage,

following guidelines set forth in Grantham et al, would result in enhanced expression.

Applicants appear to argue that since the Hoekema et al reference related to yeast, one of ordinary skill in the art would have no guidance as to how to affect plant genes. However, comparisons on codon usage were known for a number of groups, including plants. See Grantham et al. One of ordinary skill in the art would know that codons preferred by plants would have a positive effect on a sequence such as a Bt toxin, where the native sequence employs codons avoided in plants.

Applicants argue that one would not be led to use only the amino terminal end of the Bt gene based upon the cited references (Amendment A, pages 6-7, bridging paragraph). However, it is well known that only the amino terminal end of the sequence is required for pesticidal activity. In fact, truncated forms of the gene were known to be more highly expressed in plant cells. This teaching is found in the Barton et al and Vaeck et al references.

Applicants' arguments have been carefully considered insofar as they apply to the ground of rejection set forth above, but are not deemed persuasive. Thus, the method of improving the expression in a plant of a foreign protein was well within the ordinary skill in the art at the time the claimed invention was made as adequately demonstrated by the references. One of ordinary skill would have had a reasonable expectation of success in view of the primary reference which teaches alteration of expression in yeast by manipulating the sequence based upon the known codon preferences of the organism. Therefore, the claimed invention as a whole was clearly prima facie obvious over the references, in the absence of sufficient, clear, and convincing evidence to the contrary.

No claim is allowed.

An inquiry concerning this communication should be directed to Che Swyden Chereskin, Ph.D., at telephone number (703) 308-0034. Inquiries of a general nature should be directed to the Group 180 secretary at (703) 308-0196.

CSC 11/15/90
revised 12/20/90

JACQUELINE STONE
PRIMARY EXAMINER
ART UNIT 184

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